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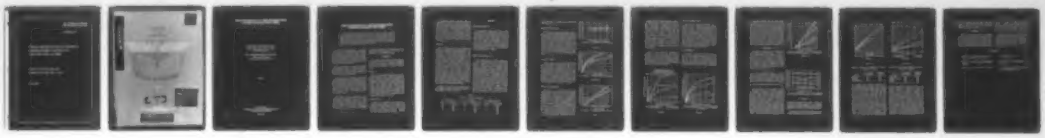

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SCHOOL OF AVIATION MEDICINE
RANDOLPH AIR FORCE BASE, TEXAS

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PAROTID GLAND SECRETION RATE AS A METHOD FOR MEASURING RESPONSE TO GUSTATORY AND MASTICATORY STIMULI IN HUMANS

HOWARD H. CHAUNCEY, Ph.D.*
*Tufts University School of Dental Medicine
Boston, Massachusetts

IRA L. SHANNON, Major, USAF (DC)†
†Dental Sciences Division
School of Aviation Medicine, USAF

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Air University
SCHOOL OF AVIATION MEDICINE, USAF
RANDOLPH AFB, TEXAS

May 1959

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PAROTID GLAND SECRETION RATE AS A METHOD FOR MEASURING RESPONSE TO GUSTATORY AND MASTICATORY STIMULI IN HUMANS

Parotid gland secretion rate was employed as a means of measuring response elicited by gustatory and masticatory stimuli in humans. A plot of secretory response against its corresponding affector intensity — bolus volume (masticatory stimuli), or application rate (gustatory stimuli) — resulted in a curve that fit the equation of Michaelis and Menton. This relationship was expressed mathematically and the maximal response (R maximum) and reflex equilibrium constant (K) for the different stimuli were calculated. The results indicated that at least two different masticosalivary and three gustosalivary reflexes were involved.

Four distinct taste sensations — acid, salt, sweet, and bitter — are generally acknowledged as comprising the gustatory sensation. These primary modalities along with certain somatosensory components such as pain, temperature, and touch contribute to the response aroused when the receptor areas of the oral cavity are activated by gustatory stimuli.

Electrical recording of action potentials from single taste fibers (1) and total receptor field response (2) has provided a means of measuring nerve response to chemical stimulation of the tongue in animals.

The "drop" and "sip" methods are generally employed to measure taste thresholds and sensitivity differentials in humans. Beebe-Center and Waddell (3) describe a heteroquantitative taste scale using the subjective strength of a 1.0 percent solution of sucrose, the units being termed "gusts." Gust concentration curves are given for tartaric acid, sodium chloride, sucrose, and quinine.

Unfortunately, these measurements of human response to gustatory stimuli are based on subjective evaluations and are thus necessarily limited in scope. Clearly some other means of exploring gustation in humans is requisite before the physiologic mechanisms governing this sensation can be established. The present paper offers a new approach to the quantitative determination of the effect of gustatory and masticatory stimuli.

Received for publication on 28 January 1959.

METHOD

Two hundred sixteen male subjects between the ages of 17 and 30 years, of sufficiently sound physical condition to qualify for military service, were utilized for the herein described study.

Stimuli

The action of masticatory stimuli was evaluated using gum base, paraffin, and rubber bands. The effect of the four gustatory submodalities was determined, using solutions of citric acid, sodium chloride, sucrose, and quinine sulfate. The solutions were applied using wetted cotton applicator sticks. One complete application (swab) consisted of running the wetted cotton around the lateral edges of the tongue followed by swabbing the entire dorsum of the tongue, from the area delineated by the circumvallate papillae to the tip.

Secretory rate

Samples of parotid saliva were obtained by means of vacuum cups (4). The secretion was collected in tubes graduated to 0.1 ml. For masticatory stimuli the mean flow-rate (ml./min./gland) was calculated by recording the time required for the secretion of 5 ml.

With gustatory stimuli, the rate was determined by measuring the volume secreted in a standard time period. Maximum stimulation time for sodium chloride and sucrose solutions was 20 minutes. Citric acid and quinine solutions were used for a maximum of 15 minutes in the collection of any single sample. However, if during the use of a gustatory stimulus a volume of 5 ml. was obtained before the maximum time was reached, the subject was permitted to terminate that sample and proceed to the next.

Sampling

Since a different group of individuals was employed for each series of experimental variables, the following standard procedure was established: After initial placement of the vacuum cup, a 10-minute acquaintance interval of stimulation was given each subject. The secretion thus obtained was considered to be a *clearance sample* and was discarded. On completion of the acquaintance session an *experimental sample* was collected. Since several successive samples were obtained from each individual, using either constant or variable test conditions, a clearance sample was collected immediately prior to each experimental sample. Only the initial clearance sample was of 10 minutes' duration, all subsequent clearance samples being limited to a 5-minute stimulation period. When gustatory stimuli were tested, the participants were given a drink of water after each experimental sample.

Sampling was done both morning and afternoon without regard for the possible effect of postprandial or diurnal variation.

RESULTS

Masticatory stimuli

Three different masticatory stimuli (gum base, paraffin, and rubber bands) were used. The amount of test material employed was standardized; gum base was used in units of the standard stick commercially available (3.0 gm.); paraffin was used in blocks ½ x ¼ x ¼ inch; rubber bands were large (size 32) pure gum rubber bands. The exact volume of each stimulus was determined by measuring its average displacement in water. The values obtained are presented in table I.

Effect of masticatory rate

The parotid gland secretory response to variations in masticatory rate was determined using a standard bolus size for each of the stimuli: gum base, ¾ unit; paraffin, 4 units; and rubber bands, 3 units. A different group of individuals was used for each stimulus. Four successive samples (at rates of 30, 60, 90, and 120 masticatory strokes per minute) were collected from all subjects. Participants were instructed to chew on the same side as the cup. A wall clock with second hand was visible to all and the masticatory rate was checked by having the subjects record their rate during the clearance and experimental samples. Variation from the desired rate averaged no more than 3 strokes per minute. Masticatory rate, for all three stimuli, was found to have only a

TABLE I

Volume of masticatory stimuli

Gum base		Paraffin		Rubber bands	
Units	Volume (ml.)	Units	Volume (ml.)	Units	Volume (ml.)
¼	0.77	1	0.60	½	0.20
½	1.54	2	1.20	1	0.40
¾	2.31	4	2.40	2	0.80
1	3.08	6	3.60	3	1.20
—	—	8	4.80	4	1.60
—	—	—	—	5	2.00

slight effect on the secretion rate of the parotid gland (fig. 1).

Effect of bolus volume

A series of experimental samples was obtained using successively larger bolus volumes. The number of successive samples collected for the gum base, paraffin, and rubber bands were four, five and six, respectively (table I). A single group of subjects was used for each type of stimulus. The results obtained are presented in figures 2 and 3. The plot of response versus bolus volume indicates that the secretory rate and logarithm of the bolus volume have a linear relationship (fig. 3). However, the slopes for the three stimuli markedly differ.

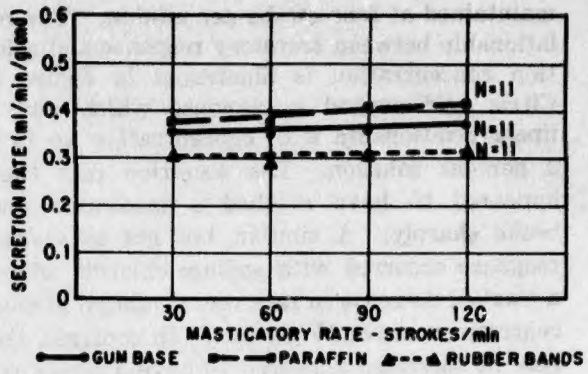

FIGURE 1

Gustatory stimuli

Three variables were studied in determining the parotid gland secretory response to the four gustatory submodalities. Effect of solution concentration, application rate, and accommodation were investigated. Twelve experimental groups were tested. A control or sham group using only water was employed to determine the response resulting from tactile sensation. The control values were subtracted from all experimental values in order to give only the response arising from gustation.

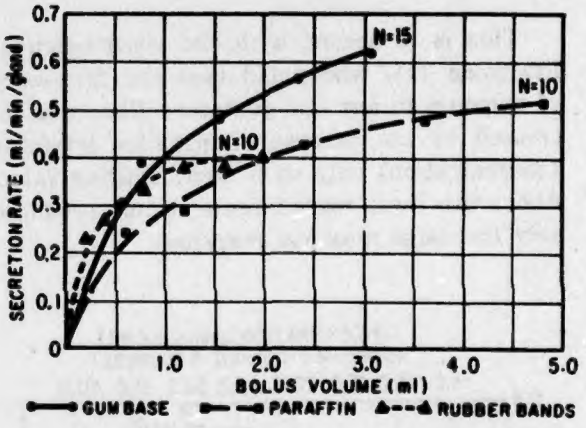

FIGURE 2

Effect of concentration

The range of concentrations of the four solutions used was chosen so that the minimal concentration would be above the absolute threshold for each sensation. The maximum concentration was selected either on a basis of solubility or on concentration compatible for continuous use without damaging the oral mucosa or inducing illness. Concentrations were always given in ascending order. Throughout the entire series of concentrations all subjects were able to discern increases in gustatory intensity from one solution to the next. A separate group of individuals was used for each of the four stimuli. Application rate was

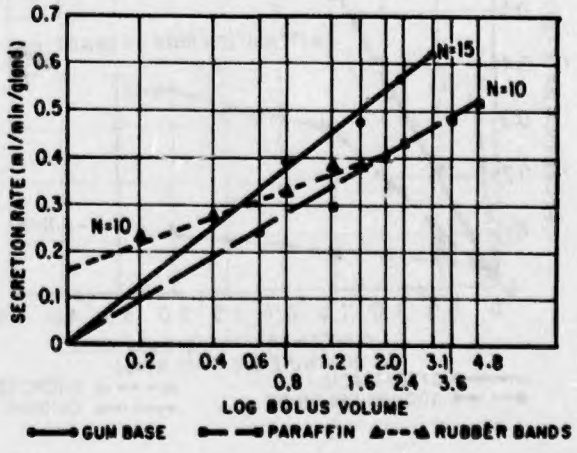

FIGURE 3

maintained at four swabs per minute. The relationship between secretory response and solution concentration is illustrated in figure 4. Citric acid evoked a response which had a linear relationship with concentration up to a 2 percent solution. The secretion rate then appeared to have reached a maximum and broke sharply. A similar, but not so severe, response occurred with sodium chloride where a marked decrease in rate was exhibited at concentrations above 15 percent. In contrast, the rate of secretion appeared to have a linear relationship with the amount of quinine and sucrose in solution throughout the entire series of concentrations.

This is in accord with the observation of Pfaffman (1), who noted that the frequency of response in any one gustatory fiber was increased by an increase of stimulus intensity (concentration) only up to some limiting value. Above this limit, more intense stimuli produced only the same maximal response.

Effect of application rate

The intensity of response resulting from increasing rates of application is presented in figures 5 and 6. Solution concentrations were constant and the rate of application was increased throughout the series. Except for quinine, the concentrations selected (citric acid, 3 percent; sodium chloride, 30 percent; sucrose, 40 percent; and quinine, 0.15 percent) were observed to induce maximal or near maximal stimulation in the concentration study. With all four stimuli, the secretory response and logarithm of the application rate appear to have a linear relationship (fig. 6).

Accommodation

In an effort to ascertain if the findings observed with increasing application rate were due to accommodation, the following was carried out. Five successive saliva samples were obtained, using (with exception of citric acid) the same solution concentrations and collection procedures as for the rate study. The lower

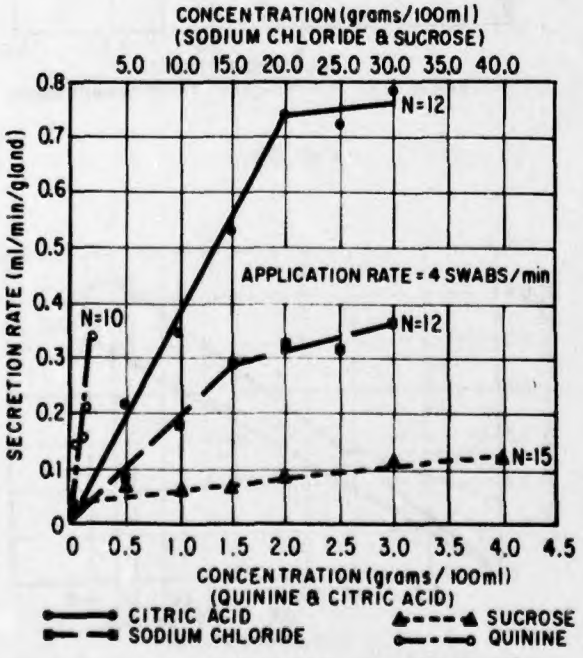

FIGURE 4

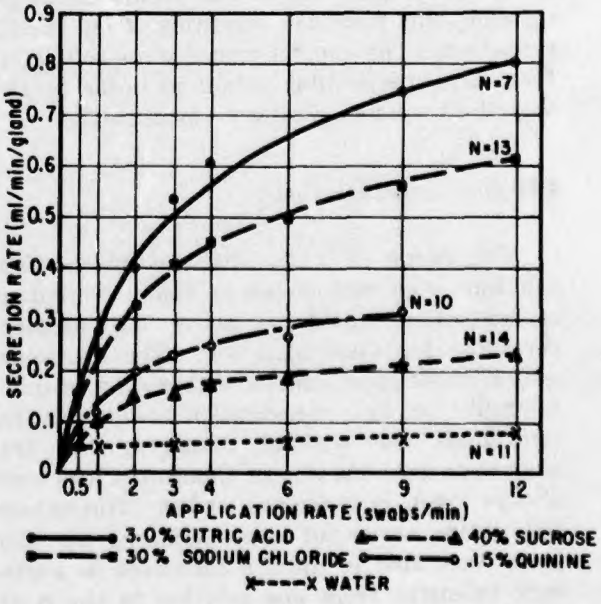

FIGURE 5

concentration of citric acid was used because concentrations greater than 2 percent had a keratinolytic action when used over a prolonged period. Application rate was maintained at 4 swabs per minute. This approximated the mean rate of 4.7 swabs per minute calculated from the range (0.5 to 12.0 per minute) of the rate series. Figure 7 shows that the response remained quite consistent over the entire stimulation period (citric acid, 80 minutes; sodium chloride, 100 minutes; sucrose, 130 minutes; quinine, 130 minutes). Thus it can be assumed that there was no measurable accommodation under the imposed experimental conditions.

DISCUSSION

Plotting the secretory response against its corresponding affector intensity — bolus volume, or application rate of gustatory stimulus— results in a curve that fits the Michaelis-Menton equation. The method of Lineweaver and Burk (5), which rearranges the equation to a straight-line function, makes the relationship between response and affecting variable more obvious. This relationship can be expressed mathematically as

$$\frac{A}{r} = \frac{1}{R}(A) + \frac{K}{R},$$

where A is the affecting variable, r is the observed response, R is the maximum response and K is the equilibrium constant of the entire reflex. By plotting A against A/r (figs. 8 and 9), the slopes of the resultant lines are equal to $\frac{1}{R}$ and the ordinate intercept is equal to $\frac{K}{R}$.

The method of the least squares was used to calculate the slopes and ordinate intercepts for the three masticatory and four gustatory stimuli. Table II contains the calculated R maximum and K for the three masticatory stimuli. The order of response intensity as represented by the R maximum is: gum base, paraffin, and rubber bands. The reflex equilibrium constants (K) for gum base and paraffin appear to be of similar magnitude while the value obtained for the rubber bands differs markedly from that obtained for the two other stimuli. The R maximum and the gustosalivary reflex K for the four gustatory modalities are presented in table III. The K for sucrose and quinine appear to be of similar magnitude and differ markedly from the values obtained for citric acid and sodium chloride.

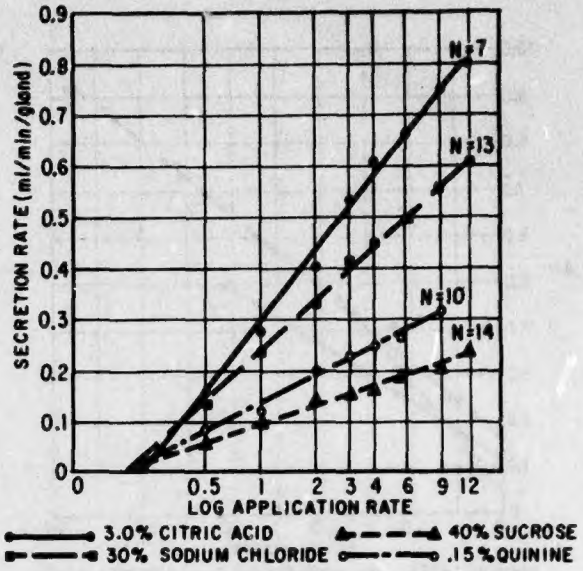

FIGURE 6

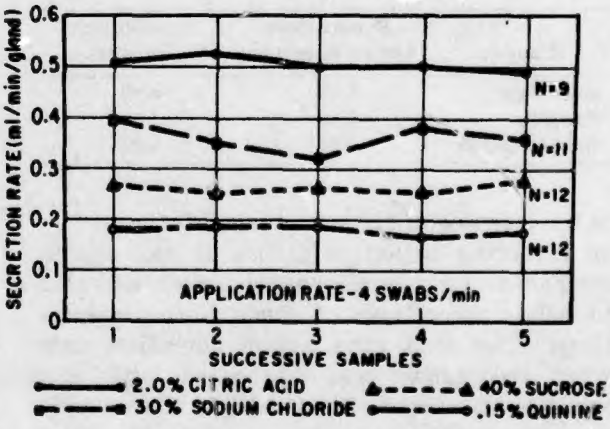

FIGURE 7

Owing to the many problems which require further clarification, we can only speculate as

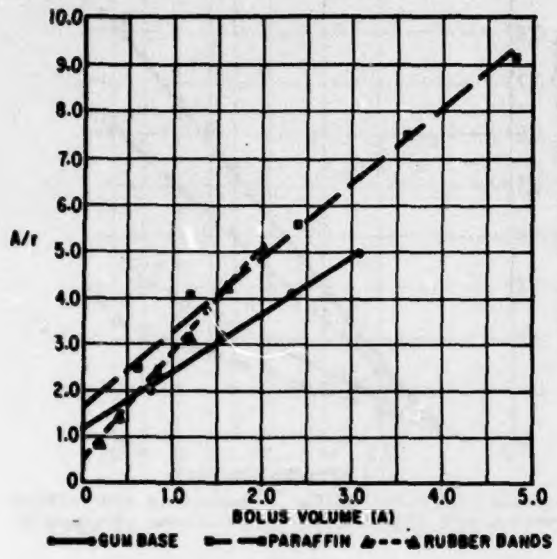

FIGURE 8

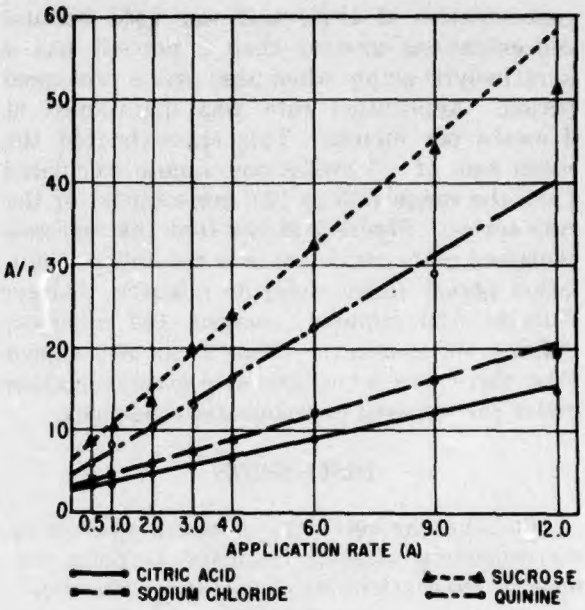

FIGURE 9

TABLE II

Maximum response and equilibrium constant of masticatory stimuli

Stimulus	R maximum (ml./min./gland)	Equilibrium constant
Gum base	0.79	0.89
Paraffin	0.62	1.00
Rubber bands	0.44	0.21

TABLE III

Maximum response and equilibrium constant of gustatory stimuli

Stimulus	R maximum (ml./min./gland)	Equilibrium constant
Citric acid	0.97	2.63
Sodium chloride	0.71	2.21
Sucrose	0.23	1.45
Quinine	0.34	1.53

to the meaning of these findings. Pfaffman (1), on recording action potentials in cat chorda tympani and glossopharyngeal nerves, was able to isolate three types of single fiber presentations. The first gave action potentials only when the tongue was stimulated with acid solution, the second responded only to acid and/or sodium chloride solution, while the third reacted to citric acid and/or quinine. Receptors for sugar were not usually found. Thus, acid was a stimulus for all gustatory nerve endings while sodium chloride and quinine affected only certain types. In essence, the three types were acid-salt, acid-quinine, and pure acid fibers. In the present investigation stimulation of the three primary modalities associated with fibers from the chorda tympani, which subserve the sensation of taste in the anterior two-thirds of the tongue (acid, salt, sweet), resulted in different responses as evidenced by the equilibrium constants (K) for the gustosalivary reflex. Although the bitter sensation is mediated, for the most part, through the glossopharyngeal nerve, the equilibrium constant for quinine was similar in magnitude to that evidenced with sucrose. These findings appear to indicate that at least three different gustosalivary reflex pathways are involved. Whether the difference is due to the type of receptor ending stimulated, or depends also on the fiber types present in the gustatory nerve trunks, cannot be discerned at this time. It is believed, however, that the

procedure described herein offers a practical means of studying the physiology of human gustation, mastication, and the mechanisms controlling salivary secretion.

SUMMARY

Parotid gland secretion rate was employed as a means of measuring gustatory and masticatory response in humans. Glandular secretion rate was observed to be a linear function of the logarithm of bolus volume (masticatory stimuli) and application rate (gustatory stimuli). Parotid flow rate was only slightly affected by chewing rate. Increasing concentrations of gustatory stimuli resulted in a secretory response which was linear throughout the entire series for sucrose and quinine, but was linear only at lower concentrations with citric acid and sodium chloride.

Mathematical expression of the relationship between glandular secretion rate, bolus volume, and application rate permitted calculation of maximal response and reflex equilibrium constants for the various stimuli. The results indicated that at least three gustosalivary reflex pathways are involved.

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